MICROORGANISMS FROM PERMAFROST VIABLE AND DETECTABLE BY 16SRNA ANALYSIS: A MODEL FOR MARS. A. I Tsapin[1], G. D. McDonald[1], M. Andrews[1], R. Bhartia[1], S. Douglas[1], and D. Gilichinsky[2], [1]Jet Propulsion Laboratory, MS 183-301, 4800 Oak Grove Drive, Pasadena, CA 91109, USA, tsapin@jpl.nasa.gov, [2]Institute of Basic Biological Problems, Russian Academy of Sciences, 142292, Pushchino, Moscow Region, RUSSIA

Introduction: Preliminary studies of Arctic and Antarctic permafrost have shown that this environment harbors microorganisms which can be isolated in pure culture, and that these organisms can survive for a long period of time (up to 20 Ma) in permafrost. It is believed that the permanent subzero temperatures in permafrost and ice environments are the main parameters ensuring the longevity of microbes. In this project we studied permafrost cores from different areas of the Siberian Arctic and Antarctic, with ages from several thousand years up to several millions years (Ma) [1, 2]. In general, Antarctic permafrost has a higher sand content, while Siberian permafrost has a texture more characteristic of clay or normal soil.

Importance of Permafrost Studies: There are several incentives in studying ancient permafrost.

1. The survivability of microorganisms for several Ma can serve as an indication of the possibility for life to be transferred from one planet to another within the solar system by impact ejecta.

2. The possibility of life to survive for several Ma gives us hope to find signs of extinct life on Mars. It also allows us to find out which biosignatures are the most resilient and long lived in the permafrost environment.

3. Comparison of the genetic material of microorganisms which have been in the frozen state for millions of years with modern organisms of the same species can give us insight into mutation rates of these organisms, and potentially aid in establishing "biological clocks".

4. Genetic material preserved in permafrost sample for hundred of thousands or millions of years may provide us an opportunity to study genomes of microorganisms that have become extinct in the surface environment.

Microbial Diversity: Analysis of Siberian permafrost samples using molecular biology methods showed that 150 clones containing 16sRNA genes could be separated into three main groups of Eubacteria. From 150 clones so far analyzed we have identified 2 species of *Arthrobacter*, 2 species of *Clostridium*, and 12 species of *Pseudomonas*.

We have not so far been able to obtain polymerase chain reaction (PCR) products from Siberian permafrost using primers specific for Archaea. However, we have recovered PCR products with primers specific for Eukarya, and phylogenetic analysis of these clones is underway.

The core from which this permafrost sample was isolated was dated using geological markers at 1.8 My old. We were able to cultivate about 20 different microorganisms from the same sample. Visual analysis of colonies showed that a large group of microorganism isolated from this sample were halophilic bacteria. Another group of organisms we have isolated from the same core were Actinomycetales.

We have also analyzed a set of permafrost samples from Antarctica (Beacon Valley). In this case we studied samples of permafrost taken from one borehole, at depths from surface to 7 meters. The deepest samples from this borehole extend below a layer of ash which was dated by several methods at approximately 8 Ma. Phylogenetic analysis of these samples is in progress. We were able to isolate just a few clones from these samples.

Environmental scanning electron microscope (ESEM) observations of permafrost samples revealed unusual bacterial cell morphologies which may in part be due to the nature of the sample itself. Drying of the sample may have contributed to the observed deviation from the coccoid or rod-like morphology expected and typical of many soil bacteria. In addition, when mineral particles were present, the cells were often physically associated with them and even, in many cases, encrusted with fine-grain minerals, indicating the close geomicrobiological associations in these samples.

Fatty acid analysis: We have analyzed the total phospholipid fatty acid composition of a 1.8 Ma old Siberian permafrost sample as shown below. We have also obtained fatty acid profiles from the Antarctic borehole samples discussed above.

Mole % fatty acids
in Siberian permafrost

Fatty acid	
11:0	<1
2OH 10:0	<1
12:0	<1
13:0	<1
2-OH 12:0	<1
3-OH 12:0	<1
14:0	9.4
i15:0	8.2
a15:0	10
15:0	8.2
2-OH 14:0	<1
3-OH 14:0	<1
i16:0	7.6
16:1d9c	6.5
16:0	20.6
i17:0	<1
a17:0	<1
17:0cyc	<1
17:0	<1
2-OH 16:0	<1
18:2d9,12	<1
18:1d9c	<1
18:1d9t	<1
18:0	8.2
19:0cyc	<1
19:0	<1
20:0	21.2

Mole % fatty acids in Antarctic permafrost

	245 (0.25m)	72 (1.3m)	281 (2.25m)	094 (4.2m)	019 (5.4m)	286 (6.2m)
Fatty acid						
11:0	<1	<1	<1	<1	<1	<1
2OH 10:0	<1	<1	<1	<1	<1	<1
12:0	<1	<1	<1	<1	<1	<1
13:0	<1	<1	<1	<1	<1	<1
2-OH 12:0	<1	<1	<1	<1	<1	<1
3-OH 12:0	<1	<1	<1	<1	<1	<1
14:0	<1	5.7	2.4	<1	<1	3.5
i15:0	<1	1.1	<1	<1	<1	<1
a15:0	<1	3.1	<1	<1	<1	<1
15:0	<1	3.6	1.2	<1	<1	<1
2-OH 14:0	<1	<1	<1	<1	<1	<1
3-OH 14:0	<1	<1	<1	<1	<1	<1
i16:0	<1	<1	<1	<1	<1	<1
16:1d9	<1	16.4	17.2	<1	7.8	3.5
16:0	100	33.3	32.9	33.3	75.9	36.9
i17:0	<1	<1	<1	<1	<1	<1
17:0cyc	<1	<1	<1	<1	<1	<1
17:0	<1	1.8	<1	<1	<1	<1
2-OH 16:0	<1	<1	<1	<1	<1	<1
18:2d9,12	<1	5.8	<1	<1	<1	<1
18:1d9c	<1	16.4	25.9	33.3	7.8	29.1
18:1d9t	<1	<1	<1	<1	<1	<1
18:0	<1	12.6	20.5	33.3	8.6	27
19:0cyc	<1	<1	<1	<1	<1	<1
19:0	<1	<1	<1	<1	<1	<1
20:0	<1	<1	<1	<1	<1	<1

Conclusions: 1. We have recovered microorganisms from Siberian permafrost which have apparently been preserved in a viable state for several millions years. This supports the possibility of interplanetary exchange of microbial life forms by meteorites such as Allan Hills 84001, which can remain in space for several millions years before they reach Earth.

2. Phylogenetic analysis of culturable microorganisms recovered after several million years at low temperatures will allow us to narrow down the search for physiologically important features of organisms that are able to survive for extended time under conditions in permafrost. Phylogenetic analysis of the organisms which can not be cultured, but which have survived and whose genetic material could be recovered, also will help us to understand mechanisms responsible for the longevity of such organisms.

References: [1] E. Rivkina et.al (1998), *Geomicrobiology, 15*, 187-193. [2] E. Vorobyova et.al. (1997), *FEMS Microbiol. Rev. , 20*, 277-290.